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Basic Investigation

Influences of electro-acupuncture at related Jing-well Points in rats with vascular dementia

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Abstract

OBJECTIVE: To observe the effects of electro-acupuncture (EA) at related Jing-well Points (HT 9, PC 9, KI 1 and LU 11) in rats with vascular dementia (VD) and discuss the relative mechanism.

METHODS: A randomized controlled animal experiment was designed. A total of 104 rats were involved in the present study and divided randomly into 4 groups: sham-operation group, model group, Jing-well Points group, and medication group. The VD model was established according to the modified 4-vessel occlusion (4-VO) method. VD rats in the Jing-well Points group were treated by EA at the related Jing-well Points (HT 9, PC 9, KI 1 and LU 11) while those in the medication group were treated with nimotop. The step-down avoidance test was performed before and after treatment in all rats. Latency and error frequency indexed memory function were recorded. Nitric oxide (NO) levels and superoxide dismutase (SOD) activity in both cerebral tissue and serum were detected after the treatment course.

RESULTS: A total of 42 rats were included in the final analysis. Compared with the model group, the latency in the Jing-well Points group was significantly prolonged (P<0.01) and the error frequency was significantly decreased (P<0.05) after therapy; the decrease in NO levels in both brain tissue and serum was significant (P<0.05 and P<0.01, respectively); and the increase in SOD activity was also significant (P<0.01). There was no significant difference in latency, error frequency, NO levels and SOD activity between the Jing-well Points group and medication group.

CONCLUSION: EA at related Jing-well Points can remarkably improve memory impairment in VD rats. Moreover, decreasing the overproduction of NO and strengthening the ability of eliminating free radicals may provide therapeutic potential for the treatment of VD.

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Key words: Electro-acupuncture; Vascular dementia; Jing-well points; Nitric oxide; Superoxide dismutase

INTRODUCTION

The development of vascular dementia (VD) is usually the cumulative effect of repeated strokes or chronic cerebral ischemia with accumulating loss of neurons. A wide variety of cognitive symptoms occur in dementia, but memory loss is the most common and indeed is a core feature of VD and any other dementia related illness. With the rapid growth of the elderly population, dementia is becoming an increasing public health burden[1,2]. Acupuncture therapy has been applied to the clinical treatment of VD and has been shown to be effective[3]. One of the hot topics in clinical and experimental studies is to find positive, effective point formulations and to explore the relative mechanisms involved. Jing-well Points are a group of specific acupoints in the category of "five shu points" in the meridian system. We have suggested that acupuncture at the related Jing-well Points would be beneficial for treating VD according to the theory of traditional Chinese medicine and relative experimental research[4]. The present study was designed to observe the effect of electro-acupuncture at related Jing-well Points (HT 9, PC 9, KI 1 and LU 11) on the memory impairment in a VD rat model and discuss the relative mechanism by observing nitric oxide (NO) levels and superoxide dismutase (SOD) activity in both brain tissue and serum.

MATERIALS AND METHODS

Experimental animals, equipment and reagents

A total of 104 adult, healthy male or female Wistar rats, ranging in weight from 200 to 220 g, were provided by the Animal Experiment Center of Anhui Medical University. The protocol was performed in accordance with ethical guidelines stated in the Guide for the Use and Care of Laboratory Animals, approved by the Committee on the Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, USA (1996).

Equipment and reagents

Experimental equipment and reagents used were as follows: The PCE-88A type programmed electro-acupuncture apparatus was purchased from the Institute of Acupuncture & Meridian (Anhui College of Traditional Chinese Medicine, Hefei, China), the 752-ultraviolet spectrophotometer and FA2104MAX210 electronic balance was from Shanghai Instrument (Group) Company (Shanghai, China), the step-down apparatus (self-made), Coomassie brilliant blue and detecting kit for NO and SOD was purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, China), and nimotop tablets (Lot No. 100071A) were from Bayer Company (Berlin, Germany).

Establishing model and grouping

A total of 104 rats had initially been trained in the step-down apparatus to learn to avoid the electric stimulus. Eleven rats were excluded due to oversensitivity or dullness. The remaining 93 rats were then divided randomly into 4 groups: sham-operation group (n=10), model group (n=29), Jing-well Points group (n=27), and medication group (n=27). The VD model was established according to the 4-vessel occlusion (4-VO) method modified by Jia[5]. The operation in the sham-operation group was similar to the rest of the groups, but both common carotid arteries and vertebral arteries were carefully kept uninjured. Not all the rats survived the operation. Therefore, 7 days after the surgery, the numbers of rats remaining in each group were 10, 12, 9 and 11 in the sham-operation group, model group, Jing-well Points group and medication group, respectively.

Treatment

Acupuncture or medication therapy was started on the 7th day after surgery. In the Jing-well Points group, the unanesthetized rats were controlled on the self-made board. Related Jing-well Points including Shaochong (HT 9), Zhongchong (PC 9), Yongquan (KI 1) and Shaoshang (LU 11) were located according to the knowledge of comparative anatomy[6]. 13 mm-long needles (30 gauge) were inserted separately into the above points approximately 2-5 mm in depth separately and connected to a programmed electro-acupuncture apparatus. A disperse-dense wave with 2-100 Hz was chosen and electric current was adjusted to approximately 1-3 mA, which resulted in slight trembling of the limbs. The acupuncture stimulus lasted 20 min and was conducted once a day for 14 consecutive days. The rats in the medication group were administrated a nimotop tablet dissolved in distilled water by gavage (20 mg/kg), twice a day for 14 consecutive days. The rats in the sham-operation group and model group received no treatment.

Behavioral testing

The step-down apparatus was a passive conditioning chamber with a size of 100 cm× 30 cm× 50 cm, partitioned into 4 compartments. The bottom of the box was made of copper grids prepared for adding an electrical stimulation. In the corner of each compartment was a wooden platform with a size of 4.5 cm×4.5 cm× 4.5 cm prepared for the rats to escape the electrical stimulation. A total of 4 rats were put separately into the 4 compartments when the training or testing began. After adaptation for 3 min, an electrical stimulation of 36 volt alternating current was added, and the animals tried to escape the injury. At first, we helped animals find the safe area and jump onto the platform to escape the electric stimulation, but the conditioned reflex developed between adding the electric stimulation and jumping onto the safe platform after repeated training and learning. When electrical stimulation was added again, the normal reaction was jumping onto the platform. Rats on the platform would jump down because of the limited space. However, they had to jump back again to escape the electric stimulation. The time from the moment the rat jumped onto the platform to the moment it first jumped down was the latency. The times that the rat jumped down and suffered the electric stimulation was regarded as the error frequency. All the rats were trained for 5 days and underwent surgery on the 6th day. Behavioral testing was performed before treatment (7 days after the operation) and after the 14-day treatment. Latency and error frequency in the 3 min indexing memory function were recorded for all rats.

Sample preparation and detection of NO and SOD activity

After the completion of the 14-day treatment, all rats were anaesthetized and 3 mL of blood sample was taken from the abdominal aorta. Following blood collection, all animals were decapitated. The right half of the brains were cut on a cryostat and weighed. Brain tissue was mixed with ice-cold physiological saline at a ratio of 1:9 and homogenates were prepared at a concentration of 100 g/L using a glass homogenizer. The homogenate was centrifuged at 3000 r/min for 15 min and the supernatant was collected for detection. NO levels and SOD activity were detected using the nitratase method and hydroxylamine method, respectively, according to manufacturer's specifications.

Statistical analysis

All data are presented as the Mean±SD and were analyzed using SPSS 11.0 Statistic Software for Windows. Mean comparison between groups was conducted using single factor variance analysis (One-way ANOVA), and pairwise comparison was performed using the least-significant difference (LSD) method.

RESULTS

Effects of EA at related Jing-well Points on memory impairment

The results of behavioral testing are shown in Table 1. Before treatment, compared with any other group, latency in the sham-operation group was significantly prolonged (P<0.01) and error frequency was significantly decreased (P<0.01). After treatment, compared with the model group, latency in the Jing-well Points group was significantly prolonged (P<0.01) and error frequency was significantly decreased (P<0.05). There was no significant difference between the Jing-well Points and medication groups (P>0.05). These results indicated the memory function of rats was impaired after 4-VO surgery and was improved by acupuncture or medication therapy. EA at relative Jing-well Points was as efficient as nimotop, which is a classic therapeutic drug for VD.

Influence of EA at relative Jing-well Points on NO levels and SOD activity

NO levels in brain tissue in the model group were significantly higher than that in the sham-operation, Jing-well Points and medication groups (P<0.01, P< 0.05, P<0.01, respectively). NO levels in serum from the model group was also significantly higher than that in the sham-operation, Jing-well Points and medication groups (P<0.01 for all). Compared with the sham-operation, Jing-well Points and medication groups, the level of SOD activity in brain tissue and serum in the model group was significantly reduced (P< 0.01 for all). There was no significant difference in the levels of NO and SOD activity in both brain tissue and serum among the sham-operation, Jing-well Points and medication groups. These results indicated that EA at relative Jing-well Points as well as medication therapy resulted in a decrease of NO levels and an increase in SOD activity in both brain tissue and serum.

Table 1 Latency and error frequency during the step-down avoidance test ($\bar{x}$ ±s)

Group	N	Latency (s)		Error frequency in 3-min (n)	
		Before treatment	After treatment	Before treatment	After treatment
Sham-operation	10	95.7±8.15	90.0±6.50	1.3±1.06	1.3±0.82
Model	12	62.0±12.28*	51.8±14.64*	3.3±0.78*	2.6±0.79*
Jing-well Points	9	60.2±11.33*	69.6±5.32*△△	3.1±0.78*	1.8±0.67△
Medication	11	62.9±5.24*	67.9±5.11*△△	2.8±0.87*	1.6±0.92△△

Note: *P<0.01, vs. the sham-operation group; △P<0.05, △△P<0.01, vs. the model group.

Table 2 NO levels and SOD activity in brain tissue and serum ($\bar{x}$ ±s)

Group	N	NO		SOD activity	
		Brain (μmol/g)	Serum (μmol/L)	Brain (NU/mg)	Serum (NU/mL)
Sham-operation	10	4.610±0.873**	7.919±0.921**	804.2±136.51**	127.8±14.56**
Model	12	6.003±0.938	9.160±0.829	671.3±118.47	95.3±15.89
Jing-well Points	9	5.122±0.884*	7.341±0.699**	812.1±115.64**	132±17.57**
Medication	11	4.903±0.803**	7.904±1.014**	802.7±129.78**	132.6±19.67**

Note: *P<0.05 and **P<0.01, vs. the model group.

DISCUSSION

Dementia scales such as the Mini-Mental State Examination (MMSE) and Hasegava Dementia Scale (HDS) have been used to assess learning and memory function of VD patients. In animal experiments, a series of behavioral tests such as the Morris water maze test, step-down avoidance test and the shuffle box test have been developed according to the conditioned reflex as the basis for studying memory, and can reflect objectively learning and memory function of animals. In the present study, the step-down avoidance test was performed. After 4-VO, latency in rats was significantly shortened and error frequency was increased. The scores of the behavioral test were sufficient to show that memory impairment had developed. The test also demonstrated that the VD model established was reliable. In the Jing-well Points group, latency prolonged and error frequency decreased, which reflected that EA at related Jing-well Points can efficiently improve memory impairment. The experimental results support the clinical application of electro-acupuncture on Jing-well Points in VD patients.

In traditional Chinese medicine, VD is classified as a form of mental retardation. Points Shaochong (HT 9), Zhongchong (PC 9), Yongquan (KI 1) and Shaoshang (LU 11) are the Jing-well Points of the heart, pericardium, kidney and lung meridians, which are used based on the concept of wholism and the theory of meridian-viscera correlativity[4]. The heart governs the circulation of blood, which is the basis of mental activity. If heart *Qi* is insufficient, blood will be deficient and cannot nourish the brain, which is responsible for the disturbance of mental activity. The kidney stores the vital essence of life and controls the growth of bone and marrow. Bone marrow has been linked to brain development, therefore, in Chinese medicine, the brain is known as the sea of marrow. If the vital essence of the kidney is insufficient, the sea of marrow will be empty and lose its mental function. The lung governs the *Qi* of the whole body and affects all vessels that can help the heart to regulate blood circulation. If lung *Qi* is insufficient, blood circulation can reduce and lead to reduced nourishment to the brain. Therefore, the heart, kidney and lung are closely related to the mental activities of the brain. Hence, these related Jing-well Points (HT 9, PC 9, KI 1 and LU 1) can be applied to treat VD.

NO is a neurotransmitter molecule synthesized from L-arginine through the activation of nitric oxide synthase (NOS). Under normal physiological conditions, NO regulates cellular metabolism, decreases cytosolic free calcium concentrations and dilates vessels by increasing cyclic guanosine monophosphate via the activation of soluble guanylyl cyclase. Therefore, NO plays a crucial role in maintaining the function of neurons, cerebral blood flow and the homeostasis of the internal environment for microcirculation. However, NO is also a free radical. Overproduction of NO can disturb normal physiological processes and turn into a neurotoxic substance. NO is involved in the development of many neurological diseases including VD[7]. During the development of cerebral ischemia-reperfusion injury, activated N-methyl-D-Aspartate receptors stimulate NOS to overproduce NO in both neurons and glia. Finally, peroxynitrite, the product of the reaction between superoxide and NO, inhibits mitochondrial respiration and induces cell apoptosis, which results in delayed neuronal death in the hippocampus[8,9]. A study by Liu et al[10]. showed that levels of NO increase in serum, the cerebral cortex and hippocampus, and mRNA expression of neuronal NOS (nNOS) increases in the cerebral cortex, hippocampus and striatum in VD rats. Moreover, NO levels in serum from 40 VD patients were observed to be significantly higher than normal subjects in a clinical study[11].

During cerebral ischemia-reperfusion injury, free radicals such as superoxide, hydroxyl radicals and NO induce lipid peroxidation in brain cellular membranes, which damage cellular protein, lipids and nucleic acids[8]. The level of SOD activity in brain tissue or serum can be detected to evaluate the ability of eliminating free radicals. In the present study, increased NO levels and decreased SOD activities were observed along side memory impairment in the model group. On the other hand, decreased NO levels and increased SOD activity resulted in an improvement of memory function in the Jing-well Points group. In conclusion, EA at related Jing-well Points can markedly improve memory impairment in VD rats. Reducing the overproduction of NO induced by cerebral ischemia-reperfusion injury and strengthening the ability of eliminating free radicals may be a possible therapeutic strategy for treating VD.

NO is produced by a family of NO synthases, which includes constitutive nNOS, endothelial NOS (eNOS) and inducible NOS (iNOS). NO produced by iNOS and nNOS is neurotoxic, while NO produced by eNOS can play an neuroprotective role[12]. The overexpression of nNOS and iNOS separately correlates with the early and late period of ischemia. Therefore, further studies should observe and distinguish the effect of EA at Jing-well Points on the different NO synthases and during different stages of VD development.

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